

Genetic Variation of the Lesser Peach Tree Borer, *Synanthedon pictipes* (Lepidoptera: Sesiidae) in Arkansas¹

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ABSTRACT The lesser peach tree borer, *Synanthedon pictipes* (LPTB), belongs to the economically important Lepidopteran family Sesiidae. No studies on genetic variation or population structure on the genus *Synanthedon* have been previously published. We examined DNA sequence variation in a 603 bp region of the mitochondrial cytochrome oxidase I gene (COI), tRNA-leu and cytochrome oxidase II gene (COII) from three LPTB populations in Arkansas. From 114 LPTB collected from three populations, a total of 53 nucleotide positions were polymorphic, and 12 distinct haplotypes were observed. The most frequent haplotype occurred in 88% of the sampled LPTB's and in all three populations. Sequence divergence among haplotypes ranged from 0.2% to 8.8%. According to the standard molecular clock proposed for lepidopteran mtDNA, the haplotypes have been diverging for up to 2.5 million years. The greatest amount of haplotype diversity was observed in the Fayetteville population where borer management is not maintained. High levels of gene flow were observed among the Clarksville, Springdale and Fayetteville populations suggesting the LPTB has a broad dispersal range. Examination of the genealogical relationships and phylogenetic analysis of the 12 haplotypes supports the existence of three genetically distinct but morphologically indistinguishable subspecies.

KEY WORDS Genetic variation, mtDNA, Sesiidae, Lepidoptera

Synanthedon pictipes (Grote & Robinson) (Lepidoptera: Sesiidae), the lesser peach tree borer (LPTB), is native to North America, and was first reported in Pennsylvania in 1868. LPTB is found east of the Great Plains and north into Canada (Taft et al. 2004). The lesser peach tree borer larvae enter trees at the barks surface where previous injury has occurred (Smith 1951). The larvae feed mostly in the trunk above soil level and within branches (Smith 1951). The larvae will pupate the following spring. The number of generations per year varies by geographic location. More northern states such as New York and South Dakota experience only one generation of LPTB per year (Smith 1951, Gilbertson 1934). Southern states such as Texas, Arkansas, Virginia and Texas have two generations per year (King & Morris 1956, Bobb 1959, Girault 1907, Wong & Cleveland 1968).

Adult emergence of LPTB also varies with geographic location. Emergence is slightly later in more southern locations when compared to the northern locations. Ohio and South Dakota emergence occurs May through September with peak occurrence in June (King 1914, Gilbertson 1934). In Virginia, Georgia,

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Texas, and Arkansas first generation emergence occurs in April through July and second generation emergence occurs early July through November (Bobb 1959, Girault 1907).

The lesser peach tree borer was first reported as a pest of plum and cherry trees. Quaintance (1906) reported the lesser peach tree borer as a pest of importance to peach trees in Georgia. LPTB causes serious damage to peach, cherry, plum, nectarine, and apricot trees. The primary economic impact is caused by girdling of limbs, which is a result of larval feeding. A gradual decline in production on damaged limbs, which when girdled will break under a fruit load and tree loss occurs from repeated girdling (Smith 1951). It is the most important indirect pest of peach and nectarine, with damage resulting in significant loss of production in orchards (Nielson 1978). LPTB feeding can also afford entry for disease organisms, eventually resulting in limb and tree death (Becker 1918). If proper borer management is maintained, sustainability of plantings is drastically increased.

Control of lesser peach tree borers in commercial orchards relies on preventing larval establishment underneath bark of trees. Once under the bark, chemical control is usually ineffective because the larvae are protected. Insecticides should be timed just before or to coincide with egg hatch. To aid in the timing of sprays, pheromone traps are used to monitor for the presence and activity of moths. Insecticidal sprays are applied 7 to 14 d after the first moths are captured in the traps because egg hatch begins about 8 to 10 d after moth emergence. If the delicate unprotected larvae are targeted, LPTB will be adequately controlled. Sprays containing Asana XL, Ambush, Lorsban, Pounce and Thiodan are labeled for control of lesser peachtree borer on peaches.

To determine the most effective time to apply an insecticide, pheromone traps should be used to monitor moth activity. These lures are synthetic copies of the chemicals female moths use to attract their mates. A trap consists of plastic top and bottom held together by a wire hanger with the lure placed inside. The inner surface of the bottom is coated with a sticky material to hold the insects once they land in the trap. Traps are hung in the tree 4 to 5 feet off the ground, usually one for each ten acres of trees in commercial orchards. In order to detect the first activity, traps should be hung in the trees well in advance of the anticipated flight. Proper identification of the moths captured in the trap is essential. There are other clearwing moths that will be attracted to the trap. Captured moths should be examined carefully to be sure that they are the correct species.

Pheromone disruption has been tested for controlling LPTB. Borers are prevented from establishing in the trunks of trees by distributing pheromone ties evenly throughout an orchard, prior to the flight of the LPTB. The ties are attached to the trees at shoulder height. If these are applied at 100 per acre then the male moths are saturated with female pheromone and cannot find the female moths. This results in no fertile eggs and thus no larvae. One application has been shown to provide control for an entire season in northwest Michigan (Thornton 2006, Gary Thornton, District Fruit IPM Agent, Larry Gut, Department of Entomology, Michigan State University). Other methods for preventing LPTB include proper orchard management. Proper pruning techniques should be maintained including thinning fruit to prevent wounds from limb breakage, being careful not to wound trees with harvesting equipment, and preventing sun scald. All wounds on the trees should be cleaned and painted with tree paint.

Dispersal is a key ecological process linking metapopulation dynamics in the landscape to distribution patterns at larger spatial scales. Dispersal can be measured via gene flow. Vandewoestijne et al. (2004) used this method to determine that the butterfly *Melanargia galathea* had a very low amount of genetic differentiation between populations, which is characteristic of species with a high dispersal capability. No information has been recorded on the dispersal capabilities of any sesiid moth.

Studying the genetic variation among populations also gives insight into the spread of insecticide resistant genes and why populations respond differently to the same control measures. Gene-flow between populations of the cotton bollworm *Helicoverpa armigera* (Hübner) in Australia was evaluated using AMOVA analysis and genetic assignment tests (Scot et al. 2005). The study characterized highly variable patterns of migration of *H. armigera* across cropping regions with some years having high migration between regions, and other years having very little migration. Development of resistance can be exacerbated in periods of low migration because there is no influx of susceptible individuals to dilute the resistance (Scot et al. 2005), but also during periods of high migration because insecticide resistant genes can be transferred rapidly between cropping regions. In years where there is little migration local management practices are of the utmost importance, but when migration increases, national management programs are the keys.

Despite the possible benefits that molecular markers may provide towards diagnostics, dispersal, insecticide resistance, and implementation of area wide control programs, none have been conducted on LPTB or any other sesiid species. The objectives of this study were to determine the extent of genetic variation within and among populations LPTB in Arkansas and to determine the amount of gene flow among populations to determine dispersal capabilities of LPTB.

Materials and Methods

Synanthedon pictipes adults were collected in 2006 using Trécé Pherocon IC wing traps (Trécé Inc., Adair, OK) baited with commercially available pheromone lures from three locations in Arkansas (Table 1): Clarksville from 2 peach orchards that did not practice borer management; Fayetteville from forests bordering an apple orchard where no insecticide pressures were encountered; and Springdale from 2 peach orchards where borer management was practiced. After specimens were collected from traps, they were identified using morphological keys (Eichlin and Duckworth 1988), and stored in glass specimen tubes at -20°C until DNA extraction. Voucher specimens are deposited at the Arthropod Museum, University of Arkansas, Fayetteville, AR.

DNA was extracted from the thoraces of individual LPTB specimens using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 μl of Tris: EDTA and stored at -20°C . Mitochondrial DNA PCR was conducted using primers C1-J-2797 (5'-CCTCGACGTTATTCAGAT-TACC-3') (Simon et al. 1994) and C2-N-3400 (5'-TCAATATCATTGATGACCAAT-3') (Taylor et al. 1997). These primers amplify approximately 606 bp of the mtDNA cytochrome oxidase I gene (COI), tRNA-leu and cytochrome oxidase II gene (COII). PCR reactions were conducted using 2 μl of the extracted DNA. The thermal cycler profile for the mtDNA COII gene consisted of 35 cycles of 94°C for

Table 1. Collection localities (latitude and longitude), sample size (n), and distance between each locality in km.

	Springdale, AR n = 31	Clarksville, AR n = 61	Fayetteville, AR n = 22
Springdale, AR			
36°11'05"N	0.00		
94°08'29"W			
Clarksville, AR	100.6	0.00	
35°28'17"N			
94°28'00"W			
Fayetteville, AR	13.7	91.2	0.00
36°03'45"N			
94°09'27"W			

45 s, 46°C for 45 s, and 72°C for 45 s per Szalanski et al. (2000). Amplified DNA from individual sesiids was purified and concentrated using minicolumns according to the manufacturer's instructions (Wizard PCRpreps, Promega). Samples were sent to the University of Arkansas Medical School Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. Accession numbers for haplotypes submitted to GenBank are EU597680–EU597691.

DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994). The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Haplotype distribution between populations, number of haplotypes, number of unique haplotypes, haplotype diversity, and average number of pairwise differences were calculated using DNAsp v4.10 (Rozas & Rozas 1999). To test for neutral mutation, the *D* statistics of Tajima (1989) and Fu & Li (1993) was calculated using DNAsp. Genealogical relationships among haplotypes were constructed using TCS (Clement et al. 2000). Tests for differentiation were conducted using AMOVA as implemented in Arlequin v 2.0 (Schneider et al. 2000).

Phylogenetic analysis was conducted using maximum parsimony analysis with the best-fitting evolutionary model as implemented in PAUP* using *Proserpinus clarkiae* (Boisduval) (Lepidoptera: Sphingidae) AF170855, was used as the outgroup taxon. Additional sesiid sequences were obtained from GenBank and from our own sequencing analysis (JAM unpublished data). Bootstrapping was performed using neighbor joining or MP (1000 replicates) to determine the reliability of the obtained topologies.

Results

DNA sequencing of a portion of the mtDNA COI gene was conducted on 114 individual LPTB sampled from three locations in Arkansas (Table 1). Among the DNA sequences, there were twelve unique haplotypes observed (Table 2). Of the

Table 2. Haplotype frequencies for 3 locations in Arkansas.

Locality	n	Haplotypes (frequency)
Clarksville, Johnson Co.	61	2, 6, 7, 8, 9, 12 (56)
Fayetteville, Washington Co.	22	1, 4 (2), 5 (2), 10, 11, 12 (15)
Springdale, Washington Co.	31	3 (2), 12 (29)

haplotypes discovered, 67% were unique to single specimens. Among the 603 nucleotide sites, 53 nucleotide sites were variable, consisting of 29 nonsynonymous and 24 synonymous mutations (Table 4). The distribution of haplotypes varied among population. Six haplotypes were observed in the Clarksville and Fayetteville populations and two haplotypes in the Springdale population. Haplotype 12 was the most frequent haplotype, occurring in 88% of the sampled LPTB's and in all three populations (Table 2). Genetic divergence ranged from 0.2% (between haplotypes 5 & 4; and 5 & 12) to 8.8% (between haplotypes 2 & 12) (Table 3).

Analysis of molecular variance based upon *S. pictipes* indicated that the within-population heterogeneity, F_{IS} accounted for 93.01% of the molecular variation with respect to haplotypes descent, and was significantly larger than the between-population heterogeneity, F_{ST} , which accounted for 6.99% of the molecular variation ($P = 0.003$). Genetic structure was evident between the Clarksville and Fayetteville populations based on Wright's F_{ST} values (Table 5). High levels of gene flow were observed ($Nm = 12.56$) between the Fayetteville and Springdale populations, which are located 8.48 miles apart (Table 5). The strongly negative values for F_u 's F_+ and Tajima's D suggest population growth in Clarksville and Fayetteville. Positive D_+ and F_+ values for Springdale suggests the population is undergoing a reduction in genetic diversity (Table 4).

A TCS spanning tree analysis was conducted on all 12 LPTB haplotypes (Fig. 1). The basal haplotype, 12, was recovered from all three populations. Haplotype 10 and haplotypes 1 and 2 formed distinct clades based on the TCS analysis (Fig. 1) This relationship among the haplotypes was also observed with the maximum parsimony phylogenetic analysis (Fig. 2).

Discussion

The Clarksville and Fayetteville populations had the greatest amount of genetic variation (Table 2). The Fayetteville population was under no insecticidal pressures and completed life cycles in wild hosts such as cherry and plum trees. The Springdale population was under high insecticidal pressures. Typically, insects under high insecticide pressure undergo a genetic bottleneck.

The LPTB's hosts include a narrow range of plants in the Rosaceae family all in the same genus *Prunus*: peach, cherry, plum, nectarine, and apricot, wild cherry and plum, along with ornamental *Prunus* species (Taft & Snow 2004). Mitter et al. (1978) studied the population genetic consequences of feeding habits of some forest dwelling Lepidoptera and found that specialized feeders have more genetic variation than generalized feeders (feeding on two or more families of host plants). They found that the occupation of two or more host did not lead to substantial genetic substructuring.

Table 3. Mean (uncorrected) distances (below diagonal) and molecular divergence* (above diagonal) for 12 haplotypes of *Synanthedon pictipes*.

	1	2	3	4	5	6	7	8	9	10	11	12
1 hap1	-	1.44E+05	2.16E+06	2.09E+06	2.16E+06	2.31E+06	2.16E+06	2.16E+06	2.31E+06	1.30E+06	2.52E+06	2.09E+06
2 hap2	0.003	-	2.31E+06	2.24E+06	2.31E+06	2.45E+06	2.31E+06	2.31E+06	2.45E+06	1.44E+06	2.67E+06	2.24E+06
3 hap3	0.050	0.053	-	2.17E+05	1.44E+05	2.88E+05	1.44E+05	1.44E+05	2.88E+05	1.59E+06	5.05E+05	7.22E+04
4 hap4	0.048	0.051	0.005	-	7.22E+04	3.60E+05	2.17E+05	2.17E+05	5.65E+08	1.66E+06	5.77E+05	1.44E+05
5 hap5	0.050	0.053	0.003	0.002	-	2.88E+05	1.44E+05	1.44E+05	2.88E+05	1.59E+06	5.05E+05	7.22E+04
6 hap6	0.053	0.056	0.007	0.008	0.007	-	2.88E+05	2.88E+05	4.33E+05	1.73E+06	6.49E+05	2.17E+05
7 hap7	0.050	0.053	0.003	0.005	0.003	0.007	-	1.44E+05	2.88E+05	1.59E+06	5.05E+05	7.22E+04
8 hap8	0.050	0.053	0.003	0.005	0.003	0.007	0.003	-	2.88E+05	1.59E+06	5.05E+05	7.22E+04
9 hap9	0.053	0.056	0.007	0.008	0.007	0.010	0.007	0.007	-	1.73E+06	6.49E+05	2.17E+05
10 hap11	0.030	0.033	0.036	0.038	0.036	0.040	0.036	0.036	0.040	-	1.95E+06	1.51E+06
11 hap13	0.058	0.061	0.012	0.013	0.012	0.015	0.012	0.012	0.015	0.045	-	4.33E+05
12 hap14	0.048	0.051	0.002	0.003	0.002	0.005	0.002	0.002	0.005	0.035	0.010	-

*based on a molecular clock for lepidopteran mtDNA (Brower 1994).

Table 4. Summary statistics for mtDNA polymorphisms*.

Locality	n	NS + S	h	δ (k)	D+	F+	D
Clarksville	61	20 + 19	6 (0.158)	0.002 (1.28)	-7.06*	-6.54*	-2.8*
Fayetteville	22	20 + 22	6 (0.537)	0.009 (5.42)	-1.85	-2.24	-2.07*
Springdale	31	1 + 0	2 (0.125)	0.0002 (0.125)	0.591	0.245	-0.77
All	114	29 + 24	12 (0.231)	0.003 (1.78)	-2.65*	-3.15*	-2.58*

*Note. n = number of sequences; NS + S = number of nonsynonymous and synonymous mutations; h = number of haplotypes (haplotype diversity \pm SD shown in parentheses); δ = nucleotide diversity; k = mean number of pairwise nucleotide differences; D+ and F+ statistics are detailed in the text (Fu and Li 1993); D = Tajima's (1989) statistic, detailed in the text. * $P < 0.05$.

Specialists are more likely to develop genetic variation due to local variation in selection coefficients due to lower migration rates in between environmental patches (Mitter 1978). Generalists could have a “homeostatic” mechanism that reduces the environmental variation perceived by loci (Mitter 1978). If specialized species lacked this mechanism chemical changes and differences among host plants could maintain genetic variation that would not be seen in more generalized species. This hypothesis could account for the amount of genetic variation observed among individuals of *S. pictipes*. Many other factors could be influencing genetic variation in this species besides host differences.

A larger amount of genetic variation was found in LPTB compared with other Lepidoptera in both haplotype number and haplotype divergence. Assuming a constant mutation rate of 2.3% per million years Brower (1994), LPTB haplotypes have diverging for approximately 72,200 – 2.52 my (Table 3). Relative to other studies on Lepidoptera genetic variation, this is a high amount of genetic divergence within a species. The haplotype divergence (0.2% to 6.1%) is larger than most variation found among other populations of Lepidoptera. Ohno et al. (2006) found 3 haplotypes of *Ostrinia nubilalis* using mtDNA with divergence 0.15% to 0.73%. Lewter et al. (2006) found that haplotype divergence among 7 fall armyworm (*Spodoptera frugiperda*) haplotypes ranged from 0.164% to 0.329%. The fall armyworm is a more generalized feeder than the LPTB feeding on plants in more than 2 families. *Ostrinia nubilalis* is a very specialized feeder and has very low divergence.

It is not surprising then, given the high amount of genetic divergence observed in LPBT, that there is genetic evidence for three subspecies of LPTB (Figs. 1–2). Figure 1 shows the 95% parsimony network in which haplotypes 1, 2, and 10 are not

Table 5. Matrix of F_{ST} (above diagonal) and N_m (below diagonal) among three LPTB populations.

Locality	1	2	3
1 Clarksville	—	0.113	0.003
2 Fayetteville	43.86	—	0.0383
3 Springdale	163.15	12.56	—

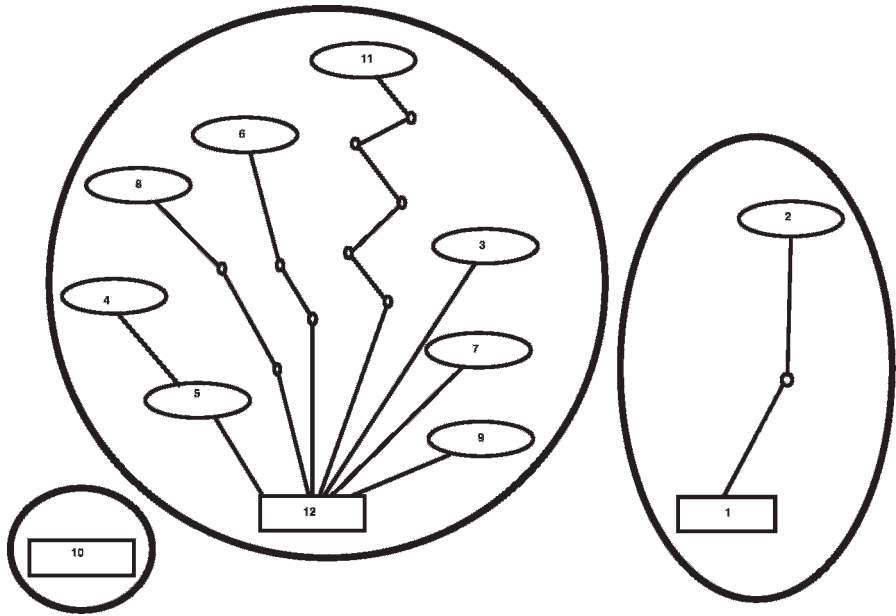


Fig. 1. Genealogical relationships among 12 haplotypes of *S. pictipes* estimated by TCS (Clement et al. 2000). A unit branch represents one mutation.

connected into the lineage. Figure 2 depicts a maximum parsimony phylogeny showing the relationship of LPTB relative to other sesiid species. Groups within LPTB formed, with one group containing haplotypes 1, 2, and 10 and the second group containing all other haplotypes. There was strong support for the distinctiveness of haplotypes 12, 1 and 2. No morphological differences were noted between subspecies and no subspecies are noted to exist. Although, 2 subspecies do occur in the closely related *Synanthedon exitiosa* (peach tree borer): *S. exitiosa graefi* and *S. exitiosa edwardsii* (Eichlin & Duckworth 1988).

Since all subspecies were collected with the same pheromone, it is unlikely that pheromone races exist in *S. pictipes*, but not altogether improbable. In Lepidoptera, pheromone races are not uncommon. Felix et al. (1996) found mtDNA variation among pheromone races of the dingy cutworm, *Feltia jaculifera* (Gn.). Studies on the genetic variation of sesiid species should be expanded. More species should be investigated especially those of economic importance. This is the first study of genetic variation among populations of a sesiid moth.

High levels of genetic variation were observed among haplotypes of LPTB in this study, however, 88% of the sampled moths belonged to a single haplotype. This gives genetic evidence that there is considerable movement of a single haplotype among populations, which is supported by the large Nm value observed.

Despite the high levels of gene flow, there is considerable evidence partitioning of haplotypes among populations. For example, Wright's F_{ST} values do show some genetic structure among populations (when values of F_{ST} are above 0.05) and this value was the largest between the Fayetteville and Clarksville populations. Also, while there was one haplotype that was very

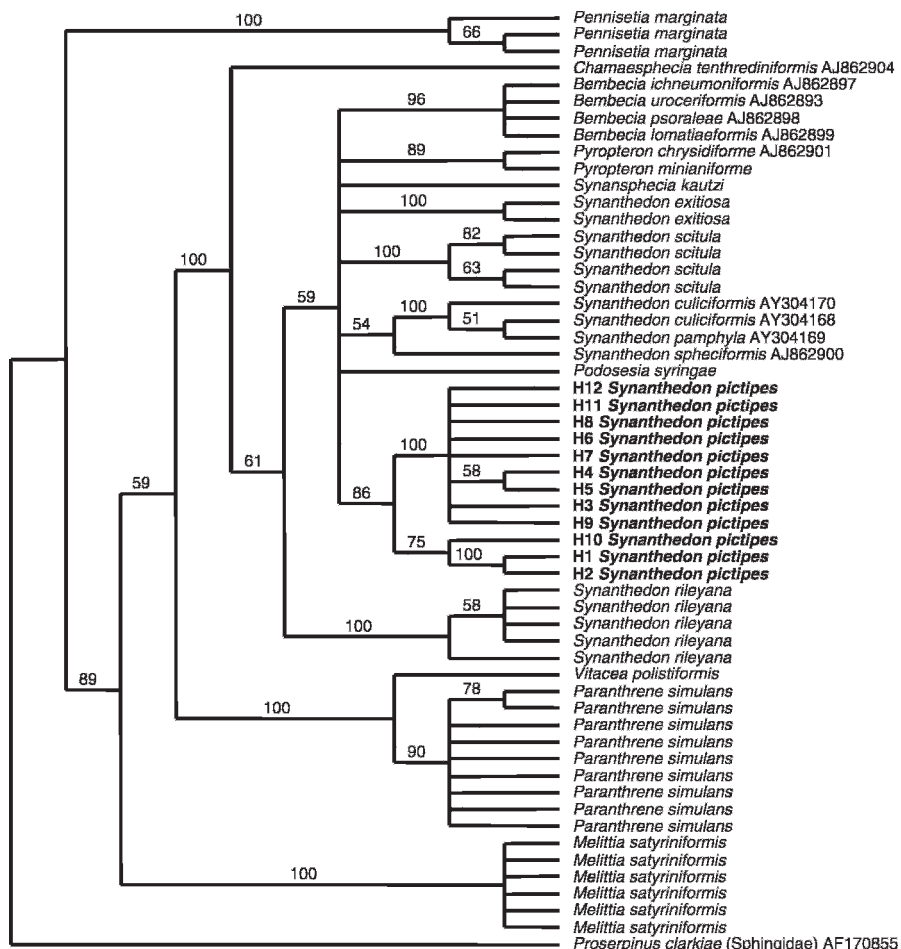


Fig. 2. Phylogenetic relationships among 12 haplotypes of *S. pictipes* relative to 19 other species of Sesiidae calculated by MP in Paup* 4.0b10 (Swofford 2001).

frequent, the majority of the haplotypes was singleton (occurring only once) and was not found among populations. This in combination with the TCS genealogical spanning tree's support for three distinct lineages, and the large amount of genetic divergence among haplotypes gives considerable genetic support for multiple morphologically identical subspecies of LPTB. Based on these observations, it is evident that considerable genetic research remains to be conducted on sesiids in order to obtain a better understanding of the evolutionary relationship among populations.

Future studies should include the use of nuclear markers to confirm the existence of subspecies, a broader sampling methodology and the use of modified pheromone traps to determine if there are pheromone races in the lesser peach tree borer.

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